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1 **Dermal uptake of nicotine from air and clothing: Experimental verification**

2

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15

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17

18 **ABSTRACT**

19 The current study aims to elucidate in greater detail the dermal uptake of nicotine from air or from

20 nicotine-exposed clothes, which was demonstrated recently in a preliminary study. Six non-smoking

21 participants were exposed to gaseous nicotine (between 236 and 304 $\mu\text{g}/\text{m}^3$) over 5 h while breathing

22 clean air through a hood. Four of the participants wore only shorts and two wore a set of clean clothes.

23 One week later, two of the bare-skinned participants were again exposed in the chamber, but they

24 showered immediately after exposure instead of the following morning. The two participants who

25 wore clean clothes on week one, were now exposed wearing a set of clothes that had been exposed to

26 nicotine. All urine was collected for 84 h after exposure and analysed for nicotine and its metabolites

27 cotinine and 3OH-cotinine. All participants except those wearing fresh clothes excreted substantial

28 amounts of biomarkers, comparable to levels expected from inhalation intake. Uptake for one
29 participant wearing exposed clothes exceeded estimated intake via inhalation by >50%. Excretion
30 continued during the entire urine collection period, indicating that nicotine accumulates in the skin
31 and is released over several days. Absorbed nicotine was significantly lower after showering in one
32 subject, but not the other. Differences in the normalized uptakes and in the excretion patterns were
33 observed among the participants. The observed cotinine half-lives suggest that non-smokers exposed
34 to airborne nicotine may receive a substantial fraction through the dermal pathway. Washing skin and
35 clothes exposed to nicotine may meaningfully decrease exposure.

36

37 **Keywords:** *Exposure Pathway, Biomonitoring, Indoor Environment, Smoking, Skin, Metabolism*

38

39 **PRACTICAL IMPLICATIONS**

40 Dermal uptake of nicotine from the air in environments with smoking or vaping can continue for a
41 substantial time after exposure. Wearing clean clothes substantially reduces uptake, but wearing
42 clothes exposed to nicotine can further increase uptake. Showering shortly after exposure may reduce
43 uptake.

44

45 **1. INTRODUCTION**

46 Exposure to nicotine via dermal contact has been widely studied, with focus on green tobacco leaves
47 and transdermal patches.^{1,2} Inhalation is typically the only pathway considered when evaluating
48 nicotine exposure resulting from passive smoking. Dermal uptake of nicotine from air may however
49 be an important pathway of exposure among passive smokers, including children. Recent modeling
50 suggested that dermal uptake of certain organic compounds, including nicotine, directly from air can
51 be a significant exposure pathway.^{3,4} Weschler et al.⁵ experimentally demonstrated for the first time
52 that dermal uptake of two gas-phase phthalates, diethyl phthalate and di(n-butyl) phthalate, can be
53 comparable to or higher than intake via inhalation. Morrison et al.⁶ showed that clean clothing can

54 impede, while clothing that has previously absorbed/adsorbed indoor air pollutants can increase
55 dermal uptake. We have recently demonstrated dermal uptake of airborne nicotine directly from air
56 or from exposed clothing.⁷ The air-to-skin-to-blood pathway may also be relevant with regard to
57 thirdhand smoke, which can be associated not only with indoor surfaces, but also skin and clothes.⁸
58 Moreover, evaluating this unexplored route of exposure is all the more important in light of the
59 increasing adoption of relatively unregulated e-cigarettes.⁹ E-cigarette use results in elevated levels
60 of nicotine in air and on surfaces including clothing. Although the exposure conditions may differ
61 from environments with environmental tobacco smoke (ETS), e-cigarettes are also anticipated to
62 contribute to public secondhand exposure via the dermal pathway.^{10,11}

63
64 The effect of washing/bathing on dermal uptake directly from air has not been investigated. Hand
65 washing and showering remove skin lipids and can reduce percutaneous penetration of certain
66 compounds.¹² Washing removed on average 96% of nicotine residue from the hands of tobacco
67 harvesters.¹³ In an *in vitro* study by Zorin et al.¹⁴, pure nicotine and nicotine in various concentrations
68 in water or ethanol was removed by washing three or five minutes after application on human skin.
69 Permeation through skin continued after removing almost all nicotine from the skin surface,
70 indicating rapid development of a nicotine reservoir in the skin itself. The cumulative concentration
71 in the receptor compartment was however greatly reduced when the skin was rinsed after three
72 minutes compared to five minutes. Whether washing has the potential to reduce dermal uptake of
73 nicotine after exposure to, for example, second hand smoke is unclear.

74
75 The preliminary study of nicotine absorption from air by Bekö et al.⁷ was performed on two bare-
76 skinned and one clothed participant. Daily pooled urine samples were collected over 60 hours after a
77 3-hour exposure in a climate chamber, where nicotine was dosed by continuous smoking of cigarettes
78 using a smoking machine. The subsequent excretion of nicotine and its metabolites indicated that skin
79 acts as a reservoir after exposure to airborne nicotine. We concluded that ionization of nicotine on

80 the surface of skin or within the stratum corneum does not substantially impede uptake. The study
81 was, however, limited in extent and detail. Furthermore, the exposures occurred in a setting with very
82 high particle levels, which confounded interpretation of the results. The current study aims to expand
83 our knowledge on the dermal uptake of nicotine from air and clothing, by conducting experiments on
84 a larger number of participants, with more controlled concentrations using pure gas-phase nicotine, a
85 longer period of exposure and collection of individual urine sample over a longer time. Additionally,
86 it assesses the effect of showering immediately after exposure on the dermal uptake of nicotine.

87

88 **2. METHODS**

89 *2.1 Human participants and experimental plan*

90 A total of six male participants were exposed to nicotine to study dermal uptake to bare skin as well
91 as investigate the impact of showering and clothing. Figure 1 presents a diagram showing the overall
92 experimental design. One experiment investigated the dermal uptake of nicotine directly from air.
93 Four healthy males between 50-68 years of age (P1-P4) participated. They were exposed to air
94 containing nicotine at elevated concentration. The exposure period was five hours. The participants
95 wore only shorts and breathed clean air through a breathing hood.⁵ The participants were asked to
96 shower the night before and again in the morning of the day following exposure. One week later two
97 of the participants (P3 and P4) were again exposed in the chamber, but they showered immediately
98 after exposure. In a companion experiment, during the first week two participants (P5 and P6; age 36
99 and 50 years, respectively) wore a set of clean clothes (underpants, socks, shirt, pants and gloves)
100 comprised of cotton, polyester and rayon while being exposed to nicotine in a fashion identical to the
101 bare-skinned participants. During the second week, these participants were exposed wearing identical
102 shirt, socks and gloves that had been exposed to nicotine at an air concentration of $\sim 500 \mu\text{g}/\text{m}^3$ for 16
103 days, then $\sim 250 \mu\text{g}/\text{m}^3$ for 11 days. They wore full-length pants and underpants that had been cleaned
104 and *not* exposed. All participants were non-smokers and were not exposed to environmental tobacco

105 smoke (ETS) or other sources of nicotine during the days prior to the exposure and during the
106 subsequent urine collection period.

107

108 *2.2 Nicotine in chamber air*

109 Nicotine (Table S1) in aqueous solution (1%) was dosed using a step-motor driven syringe in a 55
110 m³ climate chamber ventilated at an air exchange rate of 0.7 h⁻¹ (the average air temperature during
111 exposure was 29.8 °C in week 1 and 27.6 °C in week 2). Droplets of the solution were delivered onto
112 a heated stainless steel plate (35 °C), which evaporated the nicotine into the air. The dosing rate was
113 1.88 mL/h. To minimize sorption of nicotine on the chamber surfaces, the walls, floor and ceiling of
114 the chamber were covered with thin polyethylene sheet. Dosing began two days prior to exposure, in
115 order to establish steady-state nicotine concentration in the chamber air.

116

117 Nicotine in the chamber air was determined by collecting 5 to 6 L of air (100-150 mL/min) on Tenax
118 TA filled stainless-steel tubes. One sample was taken every hour during exposure and seven duplicate
119 samples were collected as well. The tubes were analyzed via thermal desorption gas
120 chromatography/mass spectrometry (TD-GC/MS) according to ISO 16000-6.¹⁵ Field blanks from
121 each day of exposure were also analyzed and the nicotine concentration was in all cases below the
122 limit of detection (< 1 µg/m³) (see Supporting Information for calibration data; Figures S1 and S2).
123 Triplicate air samples (5 L of air at a flow 40 mL/min) were collected from one of the breathing hoods
124 under conditions similar to when the participants were wearing hoods. The average measured nicotine
125 concentration in breathing hoods was 3.7 µg/m³ (st.dev. = 0.58), less than 2% of the average nicotine
126 concentration in the chamber air during these experiments. Figure S3 shows an image of the exposure
127 chamber, nicotine dosing and air sampling.

128

129 *2.3 Urine collection and analyses*

130 One to two urine samples were collected immediately before the participants entered the chamber.
131 All urine was collected for 84 hours after entering the chamber. For participants P1, P2 and P5 post-
132 exposure urine samples were pooled; one pooled sample contained urine collected within the first 12
133 hours after the beginning of exposure, the second, third and fourth pooled samples contained urine
134 collected during the subsequent three 24-hour periods. For participants P3, P4 and P6 all individual
135 urine samples were collected, weighed and analyzed in order to study in greater detail the impact of
136 clothing and showering immediately after exposure. Pooled samples were also prepared for these
137 participants; they were reconstituted from the individual samples and analyzed together with the
138 pooled samples of participants P1, P2 and P5 (Figure S4). Urine samples were analyzed for nicotine
139 and two of its metabolites, cotinine and 3-hydroxycotinine (including their conjugates after enzymatic
140 hydrolyses) via LC-MS with isotope dilution quantification, as described in Bekö et al.⁷. The limits
141 of quantification (LOQ) for nicotine, cotinine and 3-hydroxy-cotinine were 0.10, 0.05 and 0.12 µg/L,
142 respectively.

143

144 *2.4 Data analyses*

145 The mass of nicotine and each metabolite excreted was determined by multiplying the absolute
146 concentration (µg/L) in the pooled samples by the corresponding urine volume (L). For each pooled
147 sample, the amounts of nicotine and its metabolites were corrected by the corresponding amounts
148 measured in the pre-samples collected before entering the chamber, scaled by the ratio of the pooled
149 sample volume to the pre-sample volume. This allows us to obtain an estimate of dose resulting from
150 the 5 hours in the chamber. This correction for background exposure is somewhat conservative, as
151 the background urinary concentrations of nicotine and the two metabolites were somewhat higher in
152 week 2 compared to week 1 (nicotine 0.18 vs. 0.05 (1/2 LOD) µg/L, cotinine 1.73 vs. 0.28 µg/L and
153 3OH-cotinine 3.89 vs. 0.51 µg/L, respectively, for participants P3-P6 who were exposed both weeks).
154 For participants P3, P4 and P6, whose individual samples were analyzed, the excretion mass rate for

155 each interval was calculated by dividing the excreted mass per urination by the elapsed time since the
156 previous urination.

157

158 The half-lives of nicotine and its metabolites were determined from the mass excreted in the last two
159 pooled samples for each participant. For participants P3, P4 and P6, half-lives were also calculated
160 from a regression of the last 48 hours of excretion rates (appropriately log-transformed). Half-lives
161 that were greater than two-times the population mean or less than zero were excluded.

162

163 The total uptake of nicotine was calculated from the excreted amounts of nicotine and its metabolites
164 using the following molecular weights (g/mol): nicotine: 162, cotinine: 176, 3-hydroxy-cotinine: 192.

165 We estimated the amount of nicotine absorbed by assuming that 90% of nicotine and its metabolites
166 are excreted via urine and that the three metabolites and their conjugates constitute 85% of
167 metabolites excreted in urine.¹⁶ We then subtracted the amount of nicotine inhaled from hood air (IU),
168 calculated by the following equation:

$$IU = BR * C_{air} * f * t = 9 \mu g \quad (1)$$

170 where BR is the breathing rate (0.7 m³/h), C_{air} is the average air concentration in the hood (3.7 μg/m³),
171 f is the fraction of inhaled nicotine that is absorbed (0.7; see Bekö et al.⁷ for details) and t is the
172 exposure time (5h).

173

174 Finally, total uptakes of nicotine were normalized first by the chamber air concentrations of nicotine
175 and then by the participant's exposed body surface area (BSA, based on the method of DuBois and
176 DuBois¹⁷). Ninety percent of BSA was used for bare-skinned participants and for participants wearing
177 fresh clothes. For participants wearing exposed clothes, we assumed that their normalized exposure
178 from air during the 5 hours in the chamber will be the same as when they were wearing fresh clothes.
179 This fraction of their uptake was normalized by 90% of BSA. The remaining fraction of the uptake
180 was attributable to the exposed shirt, gloves and socks and was normalized by 52% of BSA.¹⁸ The

181 final normalized uptake of participants wearing exposed clothes was thus determined using the
182 following equation:

$$183 \quad Uptake = \frac{M_2 - M_1}{\frac{C_2}{52\% BSA} - \frac{C_1}{90\% BSA}} + \frac{M_1}{\frac{C_1}{90\% BSA}} \quad (2)$$

184 where M_1 and M_2 are the background and hood concentration corrected absorbed dose while wearing
185 fresh clothes and absorbed clothes, respectively (μg) and C_1 and C_2 are the corresponding nicotine
186 air concentrations during the two exposure periods ($\mu\text{g}/\text{m}^3$).

187

188 The research protocol was approved by the Capital Region of Denmark Committee for Research
189 Ethics (case no. H-16018670).

190

191 **3. RESULTS**

192 The physiological parameters of the six participants and the nicotine air concentrations are
193 summarized in Table 1. The average nicotine concentration in chamber air was between $236 \mu\text{g}/\text{m}^3$
194 and $240 \mu\text{g}/\text{m}^3$ in the first week of the experiment and between $281 \mu\text{g}/\text{m}^3$ and $304 \mu\text{g}/\text{m}^3$ in the
195 second week (Figure S5).

196

197 *3.1 Excreted amounts of nicotine and metabolites*

198 Following exposure, the concentrations of nicotine and nicotine metabolites in the urine of the bare-
199 skinned participants (P1-P4) quickly increased considerably above the levels measured before they
200 entered the chamber. They excreted a significant amount of nicotine and nicotine metabolites (Table
201 1 and Figures 2 and S6). Substantial differences were observed in the net excretion patterns among
202 participants. Participant P1 excreted large amounts of nicotine and cotinine, while participant P2
203 excreted much more 3OH-cotinine than nicotine or cotinine, reflecting faster metabolism by
204 participant P2. Participant P3 excreted similar amounts of the three compounds the first exposure
205 week. In week two, when he showered immediately after exposure, he excreted substantially smaller
206 amounts of nicotine and cotinine, but not 3OH-cotinine. Participant P4, however, excreted twice as

207 much nicotine, slightly more cotinine and comparable amount of 3OH-cotinine in week 2 compared
208 with week 1, when showering did not occur immediately after exposure. Differences were also seen
209 between the two clothed participants (Figures 3 and S7). Participant P5 excreted similar amounts of
210 cotinine and 3OH-cotinine and less nicotine, while participant P6 excreted substantially more 3OH-
211 cotinine than nicotine and cotinine, both when wearing clean clothes and exposed clothes.

212

213 The excreted amounts of nicotine and the two metabolites obtained from pooled samples were
214 compared with those from individual samples for participants P3, P4 and P6 (Figures 3 and S6, and
215 Table S2). The identical trends and similar absolute values obtained by the two methods indicate that
216 the results from reconstituted pooled samples reliably represent the observed exposure and can be
217 analyzed together with the data from participants P1, P2 and P5, who collected pooled urine only.

218

219 Net excretions of the three compounds continued to increase throughout the 84 h post-exposure period
220 for all participants. Nicotine absorption associated with the chamber exposure was not completely
221 captured even after 3.5 days of urine collection. This is supported by the excretion rates shown in
222 Figure 4, especially in the case of the nicotine metabolites that exhibit delayed excretion and longer
223 elimination half-lives compared to nicotine. Excretion rates peaked 1-1.5 days after exposure began
224 (somewhat later for metabolites) and then decayed. Half-lives of the three compounds are shown in
225 Table 2 and Figure S8. The average half-lives for nicotine, cotinine and 3OH-cotinine were 28h
226 (SD14), 35h (SD 15), and 34h (SD 19), respectively. For participants P3, P4 and P6, half-lives of
227 cotinine based on total mass excreted on consecutive days (24h pools) were generally consistent with
228 those based on a regression of excretion rates from individual samples.

229

230 *3.2 Nicotine uptake*

231 The back-calculated amount of dermally absorbed nicotine (dose) varied among the participants
232 (Table 1). The average dose was 650 μg for the bare-skinned participants during week 1 (range 460-

233 820 μg). After normalization by body surface area and chamber air concentration, the average dose
234 was $1.53 \mu\text{g}/\text{m}^2/(\mu\text{g}/\text{m}^3)$ (range 1.22-1.8; Figure 5). For participants who showered immediately after
235 exposure, normalized absorbed nicotine was lower than without showering, by 52% for participant
236 P3 and 6% for participant P4. For the two participants wearing clean clothes, the amount of absorbed
237 nicotine was 25 μg and 85 μg (0.06 and $0.18 \mu\text{g}/\text{m}^2/(\mu\text{g}/\text{m}^3)$), substantially lower than for the bare-
238 skinned participants. It increased to 470 μg and 1144 μg (1.6 and $3.1 \mu\text{g}/\text{m}^2/(\mu\text{g}/\text{m}^3)$) while wearing
239 clothes (not pants) previously exposed to nicotine. The clothing was responsible for ~95% of this
240 uptake (Figure 5).

241

242 **4. DISCUSSION**

243 *4.1 Urine concentrations and absorbed dose*

244 Peak concentrations in the 12- or 24-hour pooled urine samples of the bare-skinned participants
245 (between 10 and 85 ng/ml, data not shown) were similar to those of the two bare-skinned participants
246 in Bekö et al.⁷ and comparable to levels measured among non-smokers in hospitality environments
247 before the smoking ban. Peak concentration in the individual urine samples were slightly higher
248 (nicotine: 102 ng/ml (P3), 89 ng/ml (P4); cotinine: 71 ng/ml (P3), 38 ng/ml (P4); 3OH-cotinine: 123
249 ng/ml (P3), 68 ng/ml (P4)), approaching levels measured in light smokers.¹⁹ The total absorbed dose
250 of nicotine for the bare-skinned participants in week 1 (average 650 μg) was similar to the minimum
251 uptake estimated for bare-skinned participants based on 60h excretions in Bekö et al.⁷ (570 μg). Given
252 the long elimination half-lives and the observation that metabolites are still being excreted at the end
253 of urine collection, these doses underestimate the total nicotine absorbed.

254

255 The airborne nicotine concentration was higher in the earlier study ($420 \mu\text{g}/\text{m}^3$), where the source of
256 nicotine was environmental tobacco smoke. However, exposure lasted longer in the current study (5
257 h vs. 3 h). Moreover, the absence of particles in the current study is expected to increase the fraction
258 of total nicotine in the gas-phase.²⁰ Nicotine air concentrations were higher than concentrations

259 reported for most environments where smoking occurs. They were comparable to the levels reported
260 for smoking sections of UK and German pubs and to mean levels measured in German
261 discotheques.^{21,22} The slightly higher average concentration in the second week reflects a lower rate
262 of nicotine removal by participants; only two participants were seated in the chamber compared to
263 three exposed participants in the first week.

264

265 Nicotine and metabolite concentrations measured in pooled samples for the participants wearing clean
266 clothes were low (15 ng/ml). Clean clothes are expected to be protective for compounds like nicotine
267 that meaningfully sorb to clothing fibers, reducing the rate of transport to the skin.²³ When the
268 participants wore a set of exposed clothes, the concentrations were comparable or higher than for the
269 bare-skinned participants (peak nicotine: 29 ng/ml (P5), 55 ng/ml (P6); peak cotinine: 46 ng/ml (P5),
270 43 ng/ml (P6); peak 3OH-cotinine: 48 ng/ml (P5), 148 ng/ml (P6)). These concentrations are higher
271 than observed in Bekö et al.⁷, which is probably due to a longer pre-exposure of the clothes, a longer
272 wearing time in the chamber and a larger body surface area covered with exposed clothes. The total
273 uptakes of participants P5 and P6 with exposed clothes covering only part of the body (~50%) were
274 similar or higher than uptakes of the bare-skinned (~90% exposed) participants, indicating a higher
275 uptake rate when wearing exposed clothes.⁶ Compared to the earlier nicotine study, the clothing in
276 the current experiment had been exposed to elevated concentrations of nicotine for a longer time, and
277 had likely come much closer to equilibrium with nicotine in the chamber air.

278

279 *4.2 Accumulation in skin and cotinine half-lives*

280 Excretion of nicotine and its metabolites (above generally observable background levels) continued
281 throughout the entire period of urine collection. This observation supports earlier conclusions that
282 skin acts as a reservoir for chemicals that accumulated during exposure and delivers them into the
283 blood after exposure.^{7,24,25} Comparison of background nicotine and metabolite concentrations
284 measured in the pre-exposure samples collected in weeks 1 and 2 further supports this hypothesis.

285 The average concentrations of nicotine, cotinine and 3OH-cotinine in the pre-exposure samples in
286 week 1 were <LOD, 0.28 and 0.51 µg/L, respectively. During week 2 they were 0.18, 1.73 and 3.89
287 µg/l, respectively. None of the individual background levels in week 1 was higher than the
288 corresponding value from week 2. The slightly higher pre-exposure levels in week 2 may be due to
289 somewhat higher background exposures during the days prior to entering the chambers in week 2 (for
290 which we have no indication). More likely, the higher starting concentrations reflect metabolism and
291 excretion of residual nicotine present in the body one week after the first exposure. Additionally, the
292 ratio of week 2 to week 1 background urine concentrations were higher for the bare-skinned
293 participants (range 1-18) than for the clothed participants (1-5), possibly due to the much lower
294 exposure of the latter participants during week 1.

295

296 The observed cotinine half-lives are similar to, but somewhat larger than those of non-smokers
297 exposed to ETS (Table 2 and Figure S8). Smokers take in most of their nicotine by inhalation and
298 have cotinine half-lives of ~16 h.²⁶ Exposure to nicotine in airborne ETS results in a much longer
299 half-life (27h).²⁶ The average dermal-only half-life, observed in the current study is 35 h based on
300 pooled samples and 33h based on individual samples (P3, P4 and P6). These values suggest that
301 exposure of nonsmokers to nicotine in airborne ETS is from a combination of inhalation and dermal
302 absorption, since the resulting half-life is between that for mainstream smoking and dermal
303 absorption. However, given the small number of participants, coupled with the variability of the
304 measured half-lives, these results should be interpreted with caution.

305

306 *4.3 Comparison with inhalation uptake*

307 We can estimate what the inhalation uptake during the 5h chamber exposure would be, had the
308 subjects not been wearing a breathing hood. Using a breathing rate of 0.7 m³/h¹⁸, the measured
309 nicotine air concentrations, and a value of 0.7 for the fraction of inhaled nicotine absorbed,⁷ the
310 inhalation uptake is between 580 and 750 µg, depending on the nicotine concentration in the air on

311 the day of exposure. These doses are comparable to the observed dermal uptakes of the bare-skinned
312 participants in week 1 (average 650 µg, Table 1). However, the 5 h exposure time is too short for
313 dermal uptake from the gas-phase to reach steady state.²⁷ Longer exposure time would result in
314 dermal uptake rates closer to steady-state values and larger than uptake via inhalation. Wearing
315 previously exposed clothes can further increase dermal absorption. The uptake of nicotine for
316 participant P6 was 50% higher than the corresponding inhalation uptake without a hood would have
317 been even though only about half of the participant's skin was covered by nicotine-exposed clothes.

318

319 *4.4 Differences in normalized uptake*

320 Differences were observed in the normalized uptakes between the four bare-skinned participants in
321 week 1 as well as between the two participants wearing fresh or exposed clothes. Contrary to the
322 results of our earlier studies indicating increasing dermal uptake with age for lipophilic
323 compounds,^{5,24} nicotine uptake during week 1 was the lowest for the oldest participant. However, in
324 our previous study the older of the two participants (identical to P4 in the current study) had a higher
325 normalized uptake compared with his 32 years younger counterpart.⁷ The older of the two clothed
326 participants in the current study had higher normalized uptake both with fresh and exposed clothes.
327 Age therefore cannot explain the differences in uptake between the participants. The differences could
328 have been caused to a certain extent by differences in skin type (thickness, hydration, pH, buffering
329 capacity), sweating, desquamation, lipid content and other skin conditions such as that related to
330 filaggrin gene loss-of-function mutation.^{28,29} The type of clothes worn after exiting the exposure
331 chamber may have had an effect as well. The substantial difference between the uptakes of the two
332 participants wearing exposed clothes may have been additionally influenced by the cloth-skin gap
333 (i.e., the clothes fitting more tightly on the participant with larger BSA (2.24 m² vs. 1.93 m²)).^{24,23}
334 Other parameters, such as geometry and permeability of the fabric, laundering and exposure of the
335 clothes to nicotine prior to wearing were identical for the two participants. Studies with more

336 participants are warranted to better elucidate the effect of age and clothing on dermal uptake of
337 nicotine.

338

339 Both participants P3 and P4 had a lower normalized uptake in week 2 when they showered
340 immediately after exposure in the chamber. After exiting the exposure chamber in week 1, the
341 participants donned clothing, which is anticipated to reduce absorption due to transfer of nicotine
342 from skin lipids to clothing.^{27,23} Showering in week 2 likely removed more effectively a fraction of
343 the nicotine in skin surface lipids that had not yet penetrated into the epidermis and the dermis. The
344 reduction of uptake after showering was much smaller in case of the older participant. It is plausible
345 that nicotine was absorbed more quickly from the surface of the skin, as older skin tends to be drier
346 (less ionization) and has a thinner epidermis.^{30,31} Additionally, we did not control for the duration of
347 showering, water temperature and soap applied. These factors influence skin dryness, skin pH and
348 consequently nicotine ionization and removal.

349

350 *4.5 Factors affecting nicotine clearance*

351 The differences among the excretion patterns of the six individuals were substantial. For example,
352 participant P2 metabolized nicotine fast and excreted more than 80% of the total (nicotine +
353 metabolites expressed as nicotine equivalents) in the form of metabolites (~50% as 3OH-cotinine),
354 while participant P1 excreted 55% as metabolites (~15% as 3OH-cotinine). Given the small number
355 of participants, we cannot reach clear conclusions regarding differences in nicotine metabolism
356 following dermal uptake. Nonetheless, some discussion of factors that influence nicotine clearance
357 seems appropriate.

358

359 The availability and activity of the enzymes responsible for nicotine and cotinine metabolism may
360 partially explain the observed differences.³² Variations in urine flow and urine pH, may also influence
361 the results. It is noteworthy that there were different excretion patterns for the same individual – in

362 week 1 participant P4 rapidly metabolized nicotine, excreting only 22% of the total excreted amount
363 as nicotine; in week 2, his metabolism of nicotine was slower (36% excreted as nicotine); in the
364 previous study, the same individual (participant 1 in Bekö et al.⁷) metabolized nicotine even more
365 slowly (44% excreted as nicotine). Nicotine is primarily metabolized in the liver, indicating that it
366 depends on liver blood flow and therefore on physiological factors such as diet and exercise.¹⁶
367 Although animal studies suggest potential metabolism to a small extent in other organs, comparable
368 human studies are lacking. We cannot evaluate the contribution from metabolism in the skin, as is
369 known to occur for other compounds (e.g., DEHP).³³

370

371 Diurnal rhythms have been shown to affect nicotine clearance. We observed peak nicotine excretion
372 rates to occur at or just before midnight (Figure S9). This is most apparent in the results of participant
373 P4, who urinated more frequently than the other participants. Hepatic blood flow falls and nicotine
374 clearance decreases during sleep. Gries et al.³⁴ modeled nicotine clearance in an experiment of 48-
375 hour constant intravenous nicotine bitartrate administration to 11 subjects for 48 hours. In contrast to
376 the results shown in Figure S9, the earlier investigators found that nicotine clearance peaked around
377 11 AM and was lowest between 6 PM and 3 AM. The difference may reflect exposure via dermal
378 absorption in the present study versus an intravenous pathway in the cited study. Gries et al.³⁴ also
379 found that eating a meal increased clearance on average by 42% at peak, which occurred one hour
380 after beginning the meal. The effect of the meal lasted nearly three hours. Taken together, circadian
381 rhythms and changes in food ingestion and urine flow may explain the diurnal excretion rates of
382 nicotine and the fluctuating ratios of individual excreted amounts of the three compounds (Figure
383 S10).

384

385 For the reasons discussed above, it is unclear whether age plays a role in the observed differences in
386 metabolism. Participants P1 and P2, with different metabolic patterns, were close in age. The older
387 clothed participant (P6) metabolized cotinine very fast and excreted nearly 60% of the total excreted

388 amount of nicotine equivalent as 3OH-cotinine both weeks of the experiment, compared to 35-50%
389 in case of the younger participant P5. However, the oldest participant P4 metabolized nicotine
390 relatively quickly in week 1, although he metabolized it substantially slower in our earlier study.
391 Gourlay and Benowitz³⁵ did not find differences in steady-state nicotine plasma or estimated plasma
392 clearance in three age groups with nicotine patches. However, decreased clearance of nicotine has
393 been reported for subjects above 65 years compared to adults between 22 and 43 years,³⁶ perhaps
394 reflecting reduced liver blood flow.³⁷ Cotinine clearance is much slower and more dependent on
395 enzyme activity, which does not change with age.³⁸ Indeed, the range for 3OH-cotinine/cotinine ratios
396 among the participants was substantially smaller than that of the cotinine/nicotine or 3OH-
397 cotinine/nicotine ratios (Figure S10).

398

399 **5. CONCLUSIONS**

400 Following our pilot study,⁷ this more extensive study supports our earlier finding that nicotine can be
401 dermally absorbed directly from air at rates comparable to or higher than via inhalation. Wearing
402 clean clothes significantly decreases short-term uptake, while wearing exposed clothes increases
403 uptake. Similar to contact exposure, nicotine absorbed dermally from air or clothing accumulates in
404 the skin and is released over a period of several days, perhaps up to a week. The cotinine half-life
405 observed in the present study, compared to cotinine's reported half-life following ETS exposure,
406 suggests that a fraction of the exposure of non-smokers to ETS may occur through dermal absorption.
407 Uptake and metabolism of nicotine after dermal exposure via air varies substantially between
408 individuals. In addition to skin condition, genetic variations in metabolic enzymes, age and diet may
409 be responsible for the variation. Washing the skin after exposure may decrease the amount of
410 absorbed nicotine. The efficacy of skin washing likely depends on a number of factors and warrants
411 further investigation. Frequent laundering of clothes that are regularly exposed to tobacco smoke or
412 nicotine from vaping is anticipated to reduce nicotine uptake through skin.

413

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419

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517 toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *J*
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519

520 **Tables and Figures**

521

522 **Table 1.** Nicotine air concentrations during exposure, net amount of nicotine and the two metabolites excreted
 523 over 84 hours after entering the chamber (corrected for background concentrations before entering the
 524 chamber), absorbed nicotine dose (corrected for background concentration and nicotine concentration in the
 525 breathing hood), and normalized uptake determined from the pooled urine samples.

Participant	Age	BSA (m ²)	Date of exposure	Aver. nicotine conc. in air ± SD (µg/m ³)	Excreted Nicotine (µg)*	Excreted Cotinine (µg)*	Excreted 3OH-Cotinine (µg)*	Estimated Dose (µg)*	Uptake normalized by adjusted BSA** & air conc. (µg/m ² /µg/m ³)
P1	50	2.16	27.9.2016	236±22	279	275	124	823	1.80
P2	51	2.07	28.9.2016	240±23	82.9	183	270	616	1.38
P3	55	1.92	27.9.2016	236±22	183	193	225	711	1.74
P3-shower			5.10.2016	281±19	90.7	91.4	172	409	0.84
P4	68	1.73	28.9.2016	240±23	78.1	129	189	457	1.22
P4-shower			4.10.2016	304±26	152	148	160	543	1.15
P5-fresh clothes	36	1.93	28.9.2016	240±23	2.4	11.2	15.4	25	0.06
P5-exposed clothes			5.10.2016	281±19	106	149	146	470	1.62
P6-fresh clothes	50	2.24	27.9.2016	236±22	14.7	18.0	48.1	85	0.18
P6-exposed clothes			4.10.2016	304±26	192	215	584	1144	3.10

526 * background corrected (see section 2.4)

527 ** 90% BSA was used for bare-skinned participants and for participants wearing fresh clothes. For participants wearing
 528 exposed clothes, equation (2) was applied to normalize by adjusted BSA.

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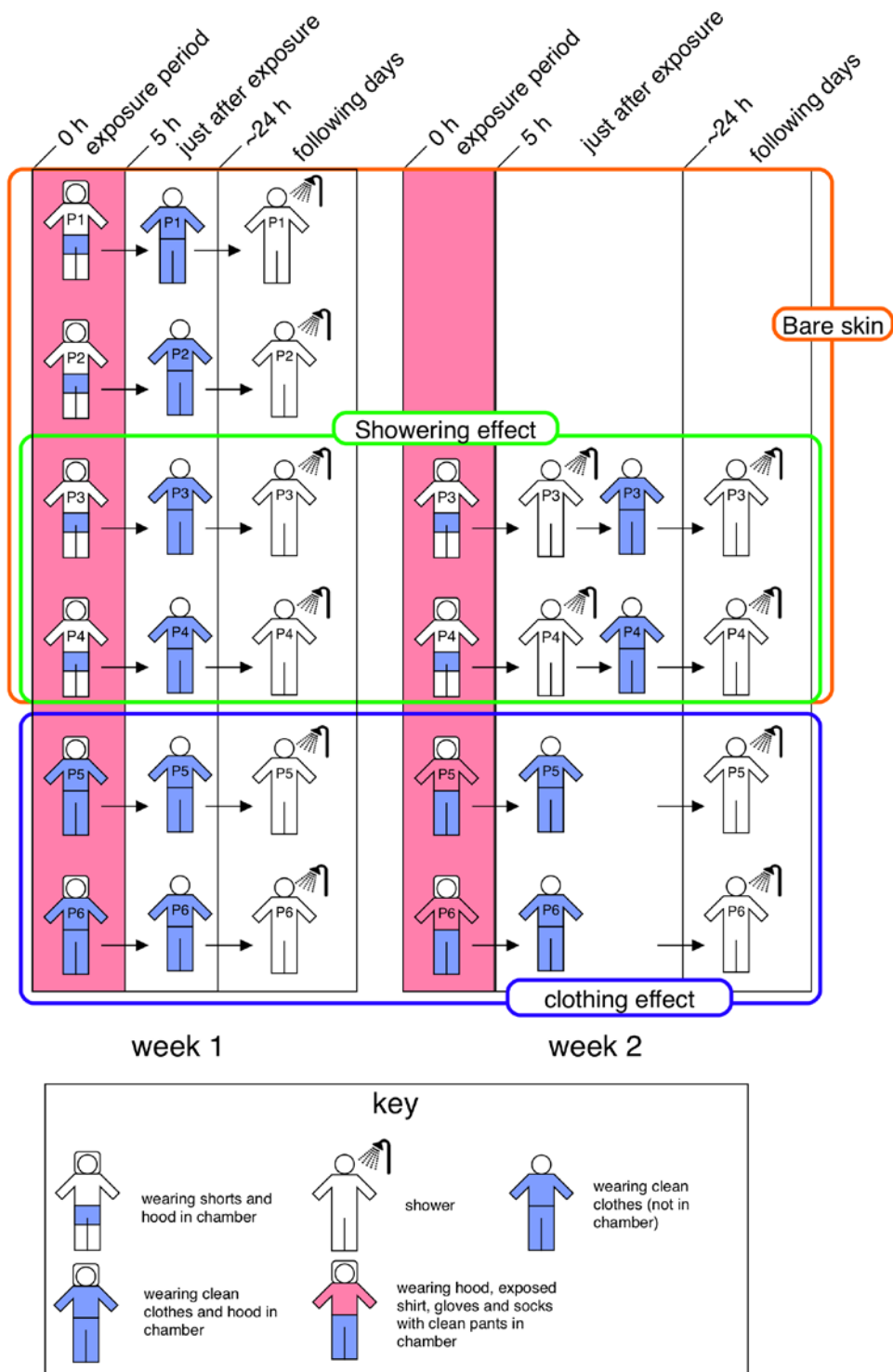
531 **Table 2.** Half-lives (h) of nicotine, cotinine and 3OH-cotinine based on last two consecutive 24-h excretion
 532 rates (pooled samples) and successive-urination excretion rates (individual samples; P3, P4 and P6 only).

Participant	Nicotine	Cotinine	3OH-cotinine
P1	34	*	*
P2	54	25	26
P3	9 (12)	19 (23)	43 (*)
P3-shower	37 (25)	27 (40)	17 (22)
P4	38 (33)	33 (39)	17 (22)
P4-shower	24 (23)	43 (34)	38 (40)
P5-fresh clothes	21	42	55
P5-exposed clothes	25	51	72
P6-fresh clothes	*(*)	17 (20)	17 (19)
P6-exposed clothes	11 (12)	62 (42)	22 (23)
Average; SD	28; 14 (21; 9)	35; 15 (33; 9)	34; 19 (25; 8)

533 * negative or unrealistically large

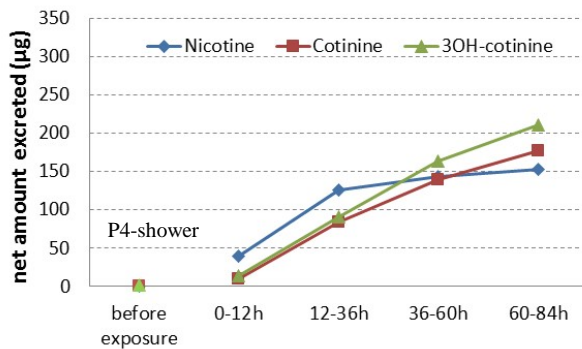
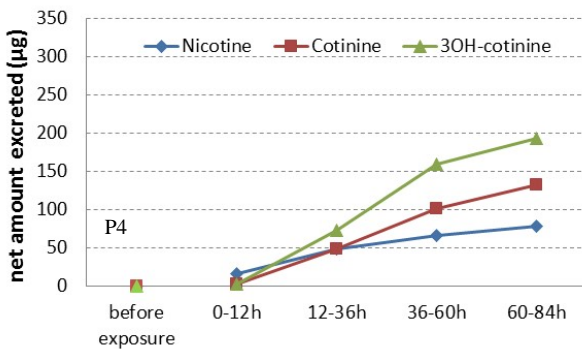
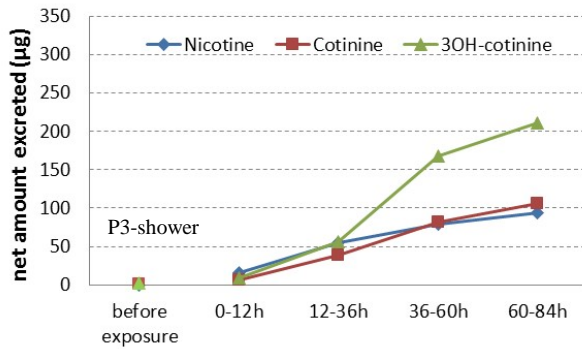
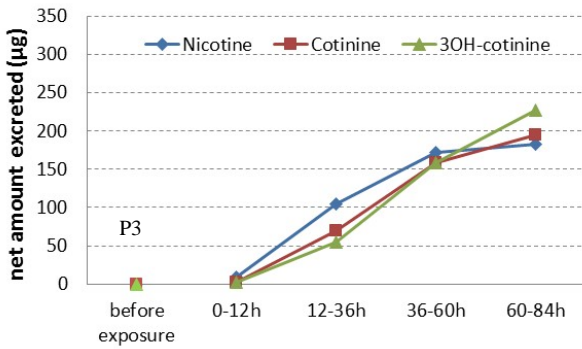
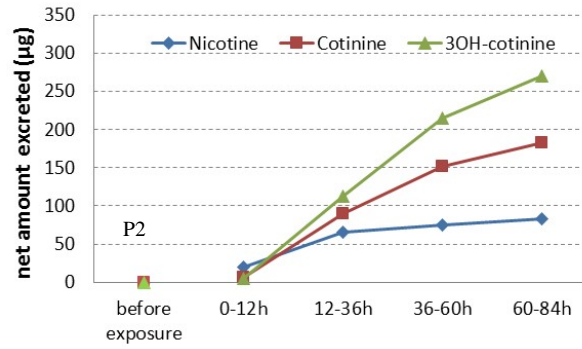
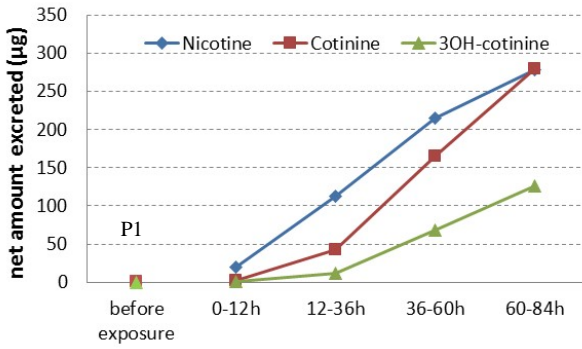
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Figure 1. Experimental plan



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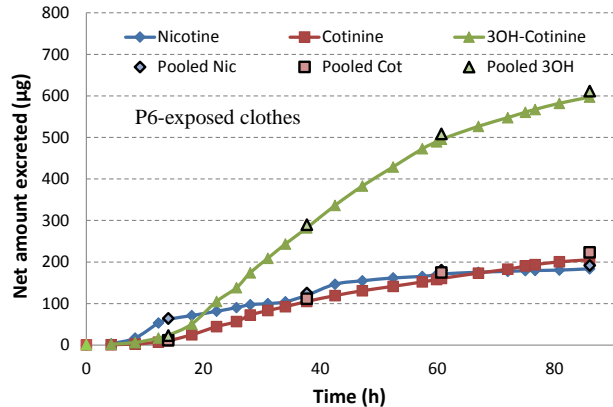
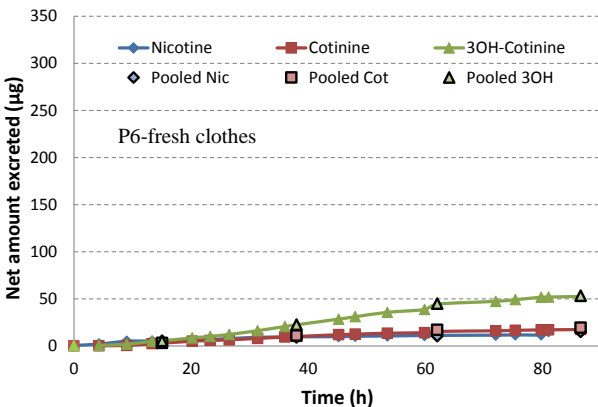
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Figure 2. Net amount of excreted nicotine and the two metabolites for the four bare-skinned participants (P1-P4). Participants P3 and P4 showered immediately after exposure on the second week (right), but not the first (left).



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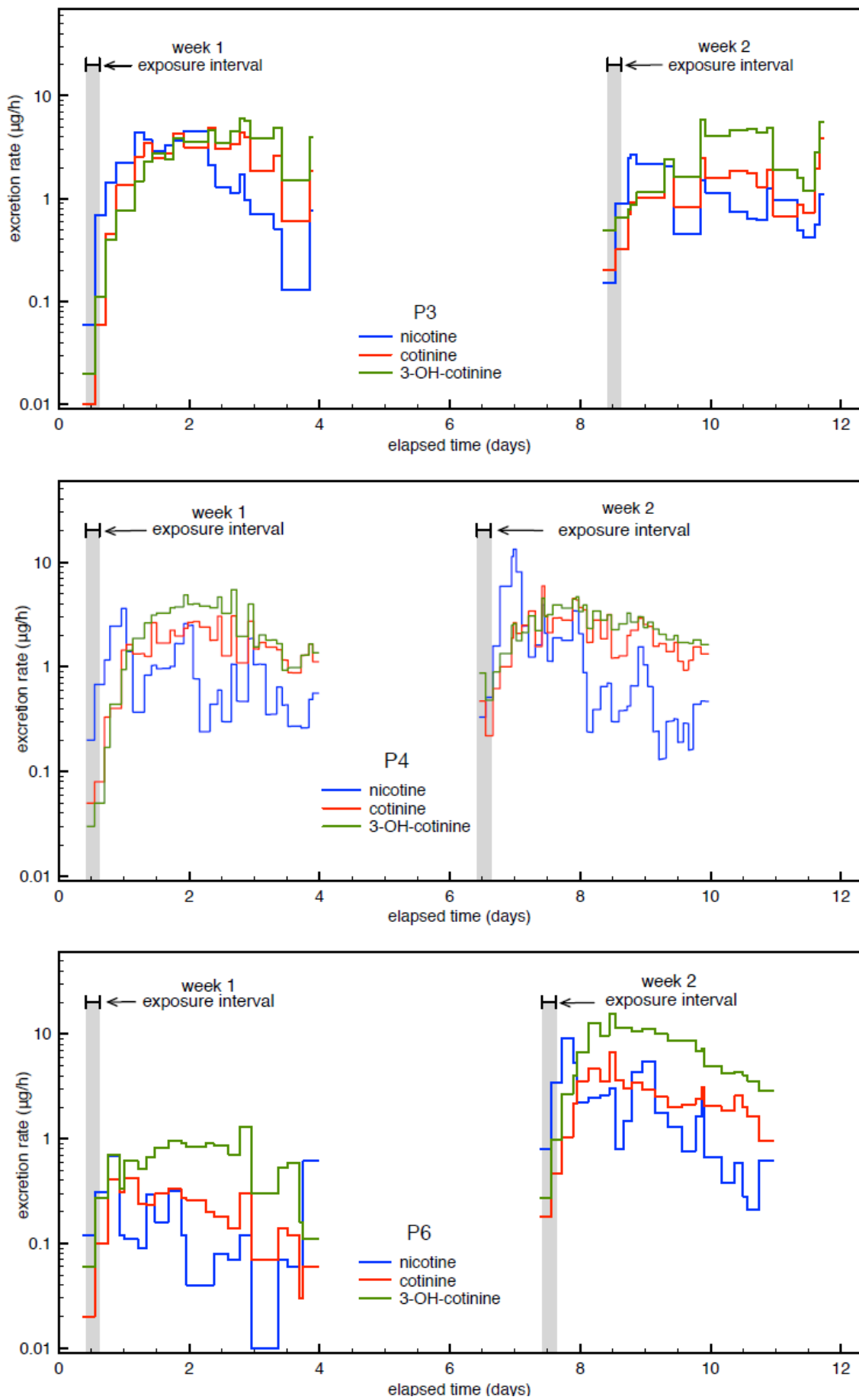
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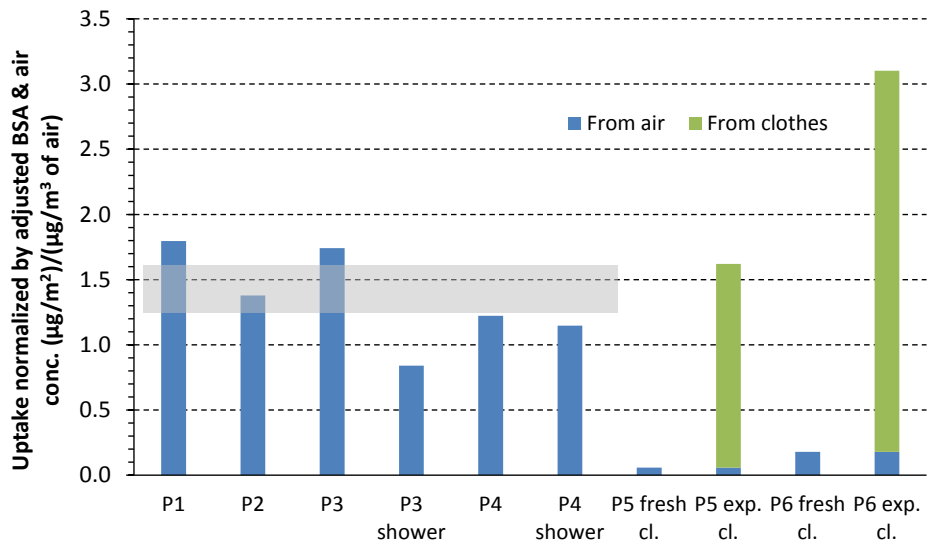
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Figure 3. Net amount of excreted nicotine and the two metabolites for one of the clothed participants, P6. Data from both the individual and pooled urine samples are shown for comparison. Note the different scales on the vertical axis. (See the Supporting Information for this comparison for the other participants.)



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Figure 4. Urinary excretion rates of nicotine and its two metabolites for participants P3 (bare-skinned), P4 (bare-skinned) and P6 (clothed).



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Figure 5. Dermally absorbed nicotine normalized by chamber air concentration and adjusted body surface area. The grey horizontal bar indicates the range of inhalation intake for participants P1-P4, normalized by air concentration and corresponding BSA (90% of total BSA).